UNIVERSITY OF GONDAR

FACULTY OF VETERINARY MEDICINE

A STUDY ON SERO-PREVALENCE OF BOVINE BRUCELLOSIS IN AND AROUND GONDAR TOWN, NORTH GONDAR, NORTH WEST ETHIOPIA

DVM THESIS

BY

ELIAS ALEHEGN

JUNE, 2015

GONDAR, ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, University of Gondar in partial fulfillment of the requirements for the degree of Doctor of Veterinary Medicine

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LIST OF ABBREVIATIONS

$	United States dollar
%	Percent
°C	Degree Centigrade
°E	Degree East
°N	Degree North
µl	Micro-liter
µm	Micro-meter
2ME	2-Mercaptoethanol
a.m.s.l.	above mean sea level
B.	Brucella
CDC	Center for Disease Control and Prevention
CFT	Complement Fixation Test
CO_2	Carbon Dioxide
CSA	Central Statistical Agency
DNA	Diribonucleic Acid
DSA	Dipstick Assay
ELISA	Enzyme Linked Immunoserbent Assay
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
H_2S	Hydrogen Sulphide
I-ELISAs	Indirect Enzyme Linked Immunosorbent Assays
MoARD	Ministry of Agriculture and Rural Development
OIE	Office International Des Epizootis
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
RFM	Retained Fetal Membrane
S19	Strain 19
SAT	Serum Agglutination Test

LIST OF ABBREVIATIONS

SPSS	Statistical Package for Social Science
U.S.A.	United States of America
WHO	World Health Organization

ACKNOWLEDGEMENT

Above all my deep and heartily praise should go to my Savior Jesus Christ for His mercy, guidance and care of my life.

Then I want to express my grateful gratitude to my advisor Dr. Shimels Tesfaye for his great and valuable support in this work.

My thanks shall also extend to Dr. Mersha Chanie for his support especially in this work and during my study period.

Though it is not enough I would like to say my God shall bless you Elifneh Tesfu (Gashe) with his family and ministers and bodies of “Healing Water International Church” for their treatment as their son and Man of GOD.

I want to say thank you to Dr. Jayne Wright for her support and hearty advice. Finally I want to thank my family for their support during my study period in memory of my beloved grandfather Alamiraw Kassa whom I have lost a week before.

ABSTRACT

Brucellosis is an infectious bacterial zoonotic disease caused by member of genus *Brucella*. The disease affects both animals and human resulting in a serious economic loss in animal production sector and deterioration of public health. This cross sectional study was conducted from November 2014-April 2015 to determine the sero-prevalence and associated risk factors of bovine brucellosis in and around Gondar town. Before serological study 47 dairy farms were selected using simple random method and a questionner survey was conducted to determine the prevalence of abortion and retained fetal membrane at the farm level. Out of 47 farms 5 were encountered with abortion and retained fetal membrane with an overall abortion prevalence of 10.6%. In the questioner survey risk factors breed, production system, herd size and breeding system were not stastically significant ($p>0.05$) but there was a difference in the prevalence among the risk factors. Cross breeds kept under intensive production system found in a herd size group of <10 animals and bred with artificial insemination had higher prevalence of 8.5%, 8.5%, 6.4% and 6.4%, respectively. After questioner survey sero-prevalence study of bovine brucellosis was carried out in the area using screening test (RBPT). Out of 406 serum samples collected using simple random sampling method 20 were found positive for RBPT with an overall sero-prevalence of 4.9%. Risk factors breed, herd size, production system, parity and abortion history were considered and analyzed. The risk factors, breed (χ^2=10.645, p=0.005), parity (χ^2 =8.766, p=0.012), production system (χ^2 =6.612, p=0.037) and breeding system (χ^2 =8.471, p=0.014) were found statically significant ($P<0.05$). Both the questioner survey and sero-prevalence study showed that there is an increment of bovine brucellosis at the study area especially at small holder farms, in cross breds, kept under intensive management system and breed using artificial insemination.

Key words: *Brucellosis, Cattle, RBPT, Sero-prevalence, Gondar, Risk factor*

1. INTRODUCTION

The majority of the world's estimated 1.3 billion poor people live in developing countries where they depend directly or indirectly on livestock for their livelihoods (World Bank, 2008 and FAO, 2009). Globally, livestock contributes about 40 percent to the agricultural gross domestic product (GDP) and constitutes about 30 percent of the agricultural GDP in the developing world (World Bank, 2009). These estimates highlight the important contribution of livestock to sustainable agricultural development. The contribution of livestock to the world's food supply, family nutrition, incomes, employment, soil fertility, livelihoods, transport and sustainable agricultural production continues to be a subject of significant review and debate (LID, 1999; ILRI, 2002; Chilonda and Otte, 2006; Thornton *et al*., 2006; Perry and Sones, 2007 and Randolph *et al*., 2007). There is no way to reach the goal of doubling of food production by 2050 without making livestock production more efficient, but this must be achieved while at the same time reducing the negative impacts of livestock products on human health and livestock on the environment (Freeman *et al*., 2007).

Livestock produce about 30% of the agricultural gross domestic product (AGDP) in the developing world, and is one of the fastest growing agricultural subsectors. Population growth, urbanization, and most importantly, increasing income have resulted in a rapid increase in demand for livestock products, which is likely to continue well into the future (Swanepoel *et al.*, 2010).

Livestock plays an important role in Ethiopian agriculture. The sector has been the focus of a breadth of analysis by experts, development partners and others that reflect a range of perspectives. The reports reaffirm that livestock continues to be a significant contributor to economic and social development in Ethiopia at the household and national level. On a national level, livestock contributes a significant amount to export earnings in the formal market (10 percent of all formal export earnings, or US$ 150 million per annum) and the informal market (perhaps US$ 300 million per annum). Moreover, livestock accounts for 15 to 17 percent of total GDP, and 35 to 49 percent of agricultural GDP. At the household level, livestock contributes to the livelihood of approximately 70 percent of Ethiopians (GebreMariam *et al.*, 2010).

Though Ethiopia has huge livestock resources in Africa, it is the untapped resource. The reasons of under-utilization are multi-factorial. These include wide spread infectious and parasitic diseases, poor management system and unimproved genetic makeup coupled with poor nutrition and malnutrition and absence of well developed market infrastructure (Degefa *et al.*, 2011).

One of the infectious diseases which are a major constraint for animal production is brucellosis. Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide. The economic and public health impact of brucellosis remains of concern in developing countries (OIE, 2000). Brucellosis results from infection by various species of Brucella, a Gram negative, facultative intracellular coccobacillus or short rod in the family Brucellaceae (IOWA, 2007). The geographical distribution of brucellosis constantly changes as new foci emerge or re-emerge. The disease occurs worldwide in both animals and humans, except in those countries where bovine brucellosis has been eradicated. In Ethiopia, serological studies of brucellosis have been carried out in farm animals. The presence in livestock varies between different parts of the country. Only few serological studies of brucellosis have demonstrated the occurrence of the disease among Borana and Hamer pastoralists; however these have highlighted the public health significance (Gumi *et al.*, 2013).

The General objectives of this cross-sectional study are;

- To know the sero-prevalence of Bovine Brucellosis in and around Gondar town.
- To know the risk factors and their effect associated with the disease occurrence.
- To give recommendations for the stakeholders on prevention and control of the disease.

2. LITERATURE REVIEW

2.1. Brucellosis

Brucellosis is an infectious bacterial zoonotic disease caused by member of genus Brucella. The disease affects both animals and human resulting in a serious economic loss in animal production sector and deterioration of public health. The disease is primary reproductive disease clinically characterized by abortion in the last trimester and retained placenta in the female whereas orchitis and epididymitis with frequent sterility occur in male. The means of transmission in both female and male are through ingestion and direct or indirect contact with excretion of the organisms in uterine discharge and milk of infected animals (Yohannes *et al.*, 2012).

Brucellosis is an important, zoonotic disease that leads to considerable morbidity resulting in significant loss of working days across the globe and thus perpetuates poverty. The disease is presented as an acute or persistent febrile illness with a diversity of clinical manifestations (Smits and Kadri, 2004). Various synonyms have been used for human brucellosis including Malta fever, Rock fever of Gibraltar, Cyprus or Mediterranean fever, intermittent typhoid and most frequently, undulant fever (Al Dahouk *et al.*, 2003). The incubation period varies between 14 and 120 days (Seifert, 1996). Primary clinical manifestations of brucellosis among livestock are related to the reproductive tract. In highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease (Radostits *et al.*, 2010). Retention of placenta and metritis are common sequels to abortion (Walker *et al.*, 1999). Females usually abort only once, presumably due to acquired immunity. In general, abortion with retention of the placenta and the resultant metritis may cause prolonged calving interval and permanent infertility. In humans, the disease is characterized by a multitude of somatic complaints, such as fever, sweating, anorexia, malaise, weight loss, depression, headache and joint pains and is easily confused with malaria and influenza (Sewell and Brocklesbry, 1990; WHO, 1997).

Humans can become infected indirectly through contact with infected animals or by animal products consumption. The disease is rare in industrialized nations because of routine screening of domestic livestock and animal vaccination programs. Clinical disease is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin, and the Caribbean (Lopes *et al.*, 2010). Brucellosis can be a serious economic disease. Losses due to abortion or stillbirths, irregular breeding, loss of milk production and reduced human productivity are some of the economic consequences of the disease. The reduced human productivity can hardly be measured in medical care (Nicoletti, 1982). The Centre for Disease Control and Prevention lists Brucella as a possible bio-terrorist agent. However, it has never been successfully used in this manner. The centre also classifies B. abortus, B. melitensis and B. suis as “agents of mass destruction” and as category B organisms (CDC, 2002).

In Africa, brucellosis is considered to be one of the most serious disease problems facing the veterinary profession. The high prevalence is due to close human-animal contacts, food consumption customs and the fact that many countries have not yet started control or eradication schemes. Brucellosis is perhaps the most widespread and economically important disease in tropical and sub-tropical regions. The direct loss of meat (as a result of abortion, infertility and weight loss) in infected herds of cattle was estimated to be 15% while that of milk (reduced milk production) was 20%. In Ethiopia, brucellosis is one of the infectious diseases, which causes reduced productivity as reported by few studies (Kebede *et al*., 2008).

The diagnosis of brucellosis depends on serological testing and on the isolation and identification of the infecting Brucella species. Care should be taken during collection and transportation of specimens, which should be processed in a biohazard cabinet (Quinn *et al*., 2004).

Treatment is unsuccessful because of the intracellular sequestration of the organisms in lymph nodes, the mammary gland, and reproductive organs. Brucella species are facultative intracellular bacteria that can survive and multiply within the cells of the macrophage system. Treatment failures are considered to be due not to the development of antimicrobial resistance but rather to the inability of the drug to penetrate the cell membrane barrier. Control programs have employed two principal methods: vaccination of young or mature animals and the slaughter of infected and exposed animals, usually on the basis of a reaction to a serological test (Radostits *et al.*, 2000).

2.2. Bovine Brucellosis

Bovine brucellosis is mainly caused by B. abortus; to a lesser extent by B. melitensis, and occasionally by B. suis. Clinically, it is characterized by abortion and retained fetal membrane (RFM) in cows and orchitis and epididymitis in bulls. Sources of infection include aborted fetuses, fetal membranes, vaginal discharges and milk from infected cows. The most common route of transmission in cattle is through direct contact with an aborting cow and the aborted fetus or by indirect contact with contaminated fomites. Ingestion of contaminated pasture, feed, fodder and water may also play a secondary role (Adugna *et al*., 2013).

Bovine brucellosis is characterized by reproductive failure which can include abortion, birth of weak, unthrifty calves, orchitis and/or epididymitis in male. The organism causes abortion in cattle after the fifth month of pregnancy with retention of placenta, metritis and subsequent period of infertility. The proportion of cows that abort within a herd is variable and small percentage of infected cows abort more than once (Enright, 1990). Bovine brucellosis caused mainly by B. abortus is still the most widespread form of the disease (Corbel, 1997). The disease in cattle is widely distributed and has been recorded in 120 out of 175 (68.8%) countries of the world (Nielson and Dunkan, 1990).

2.2.1. Causative Agent

Brucellosis in cattle is usually caused by biovars of B.abortus. In some countries, particularly in southern Europe and western Asia, where cattle are kept in close association with sheep or goats, infection can also be caused by B. melitensis. Occasionally, B. suis may cause a chronic infection in the mammary gland of cattle, but it has not been reported to cause abortion or spread to other animals (Lopes *et al*., 2010).

Brucellae are small, cocco-bacillary or short rods; with a size range of 0.5 to 0.7 μm. by 0.6 to 1.5 μm. These organisms are gram negative and frequently take the counter stain poorly and require a minimum of three minutes for a good definition. They are aerobic, non-motile, non fermenting and non-toxigenic. They occur singly or in groups are non-sporulating and non encapsulated (Grimont *et* *al*., 1992). Six species of pathogens are known as causative agents of brucellosis in different animals, which include B. abortus that is the causative agent of brucellosis in cattle and Bang's disease in humans. B. melitensis the causative agent of brucellosis in small ruminants and Undulating or Malta fever in humans. B. ovis, the causes brucellosis in sheep and B. suis the causative agent of brucellosis in pigs which also can be transmitted to humans. B. canis, the causative agent of brucellosis in dogs and B. neotomae occurs in desert rats in the USA. The different species cannot be distinguished from each other morphologically; however, differentiation of B.abortus, B.melitensis, and B.suis is based on quantitative differences in several physiologic tests. Such tests include requirement of increased CO_2 for growth, H_2S production and growth in the presence of basic fuschin and thionin. Besides, within each of these species of Brucella, a number of strains have been recognized on the basis of these tests and additional biochemical properties (Grimont *et al*., 1992).

2.2.2. Epidemiology

Brucellosis has a worldwide distribution and it is an important disease among livestock and people in sub-Saharan Africa. In Ethiopia, there is no documented information on how and when brucellosis was introduced and established. However, in the last two decades, several serological surveys have showed that bovine brucellosis is an endemic and widespread disease in the country (Berhe *et al*., 2007).

The geographical distribution of human brucellosis is closely related to the endemicity of animal infection, the methods of animal husbandry, human food habits, the standard of hygiene and other socioeconomic activities (Abdusalam and Fein, 1975). Thus, it is mandatory to study and establish the occurrence of this disease in the reservoir animals including cattle, swine and other animals prior to the inquiry of human brucellosis (Alton, 1973; Abdusalam and Fein, 1975). Infected animals shed organisms in the milk. Raw milk or raw milk products of bovine or caprine origin are ready sources for infections in humans. Cattle are the natural host of B.abortus and are primarily responsible for its maintenance in an animal population. Generally the transmission of Brucella species from its preferred host to a new host is a rare event and when it takes place usually results in the localization of the bacteria in the mammary gland and the reticulo-epithelial system rather than in the uterus and fetal membranes. Over the years, Brucella infections have also been documented worldwide in a great variety of terrestrial wild life species and more recently in marine mammals. It may be present in wildlife as the result of spillover of infection from domestic animals or as a sustainable infection (Hirsh *et al*., 2004).

2.2.3. Modes of Transmission and Risk Factors

The disease is transmitted by ingestion, penetration of the intact skin and conjunctiva, and contamination of the udder during milking. The organism does not multiply in the environment but merely persists, and the viability of the organism outside the host is influenced by the existing environmental conditions. Grazing on infected pasture, or consuming other feedstuffs and water supplies contaminated by discharges and fetal membranes from infected cows, and contact with aborted fetuses and infected newborn calves are the most common methods of spread (Radostits *et al*., 2010). Transmission occurs mainly by ingestion of contaminated feed and water by organisms, which are present in large numbers in aborted fetuses, fetal membranes and uterine discharge. However, infection through injured/intact skin, the mucosa at the respiratory system and conjunctiva frequently occurs. Calves can be infected in uterus or suckling of infected dams (Degefu *et al*., 2011). Brucellosis is not usually transmitted from person to person. Rarely, bacteria have been transmitted by bone marrow transplantation, blood transfusion or sexual intercourse. Rare congenital infections have also been documented. In some cases, the infant appeared to be infected through the placenta and in others by the ingestion of breast milk. Brucellosis was reported in an obstetrician who swallowed secretions while trying to clear a congenitally infected infant's respiratory tract at birth (Munir *et al*., 2008).

The risk factors that influence the initiation, spread, maintenance, and/or control of bovine brucellosis are related to the animal population, management, and the biology of disease. From a practical viewpoint, the factors influencing the transmission of brucellosis in any given geographical region can be classified into two fundamental categories: those associated with the transmission of disease between herds and those influencing the maintenance and spread of infection within herds (Radostits *et al*., 2010). Among the potential risk factors considered in the lowland area, breed, herd size, management system, mating methods and sources of replacement stock each had a significant effect on the sero-prevalence of brucellosis. The prevalence was higher in crossbred animals (10.3%) than in indigenous ones (2.7%). A higher prevalence was found in animals from smaller herds (10.2%) than in animals from medium (2.8%) and large (2.2%) herds. More positive test results were recorded in animals raised under intensive production systems (10.3%) than in those raised in extensive systems (2.7%). Similarly, a higher sero-prevalence of brucellosis was observed on farms that used artificial insemination (10.3%), as opposed to those that used natural mating (2.7%) (Jergefa *et al.*, 2009).

Comparison was made on the sero prevalence of brucellosis in male and female animals to observe the effect of sex in the abundance of the disease, the result of the study showed that the sero-positivity was higher in female animals than in male animals. Based on age groups, zero prevalence was recorded in animals with less than two years of age. Sero-prevalence of bovine brucellosis in local and crossbreed animal was 1.71% and 3.64%, respectively (Yohannes *et al*., 2012).

Susceptibility of animals to brucellosis depends on their natural resistance, level of immunity and, environmental stress. Age, sex and breed and pregnancy status of the animal is also a risk factor for susceptibility. Younger animals tend to be more resistant to infection and frequently clear infections than sexually mature ones. Mature animals are much more susceptible to infection, regardless of sex. In female animals, pregnancy has positive contribution to the degree of susceptibility than their age. Bulls are relatively resistant than sexually mature heifers and less resistant than sexually immature heifers (Degefu *et al*., 2011).

2.2.4. Occurrence in Ethiopia

This disease is endemic in Ethiopian and researchers have established its prevalence rate in cattle in different regions of Ethiopia though, countrywide prevalence in cattle is not yet determined. However, with the exception of few case reports by (Ephrem, 1981) from Bale Administrative region and also (Teshale, 1982) reported cases of brucellosis on four patients from Tigray, Sidamo, Arsi and Shoa Administrative regions by standard serum agglutination test, but the prevalence rate of the disease in human population is not studied and is unknown in Ethiopia (Kassahun, 2003).

The evidences of Brucella infections in Ethiopian cattle have been serologically demonstrated by different authors. A relatively high sero-prevalence of brucellosis (above 10%) has been reported from smallholder dairy farms in central Ethiopia while most of the studies suggested a low sero-prevalence (below 5%) in cattle under crop-livestock mixed farming. There is a scarcity of published literature on the status of cattle brucellosis in pastoral areas of the country where large population of cattle are reared. So far, a study carried out in east Showa zone of Ethiopia showed a relatively higher sero-prevalence in pastoral than agro pastoral system (Megersa *et al*., 2011).

In Ethiopia there is no documented information on how and when brucellosis was introduced and established. However, in the last two decades several serological surveys have showed that bovine brucellosis is an endemic and wide spread disease in the country. For instance, prevalence of 18.4% around Addis Ababa , 2.4% in Jimma zone, 11.6% in Sidama region, 4.2% in south east Ethiopia, 2.9% in Central Oromia , 11.2% in east Showa zone, 4.9% in Tigray region , 7.61% in Arsi region and 1.11% in Addis Ababa and Sululta abattoir were documented in different parts of Ethiopia (Yohannes *et al*., 2013).

In Ethiopia, so far higher sero-prevalence reports are 39% in western Ethiopia (Meyer, 1980), 8.2% in Arsi area (Bayleyegne, 1989) in central part of the country, 22% in a dairy farm in northeastern Ethiopia (Tariku, 1994), 8.1% in dairy farms in and around Addis Ababa (Yilkal, 1998), 11%-15% in dairy farms and ranches in southwestern Ethiopia (Tekleye *et al*., 2000), and 7.7% in Tigray region (Haileselassie *et al*., 2010). Relatively low individual animal sero-prevalence in intensive farms were recorded in different part of the country (Taddesse, 2003) observed a prevalence of 0.14% in north Gondar Zone (Tadele, 2004) reported 0.77% in southwestern Ethiopia, and (Kassahun *et al*, 2007) documented 2.46% in Sidama Zone of southern Ethiopia. Furthermore, a recent study conducted in pastoral and agro-pastoral areas of East Shoa Zones of Oromia Regional State by (Hunduma and Regassa, 2009) reported the prevalence of bovine brucellosis to be 15.2% for pastoral and 4.1% for agro-pastoral areas. Similar other studies on livestock brucellosis were done in pastoral and agro-pastoral areas of East Africa (Omer *et al*., 2000) reported 8.2% sero-prevalence in Eritrea and (El-Ansary *et al*., 2001) reported 5% in Sudan. So far there is no published data on bovine brucellosis for agro-pastoral areas of Somalia Regional State (Degefu *et al*., 2011).

2.2.5. Zoonotic Importance

Brucellosis is an important zoonosis causing undulant fever in humans. The disease is also called Undulant Fever, Malta Fever, Mediterranean fever, Enzootic Abortion, Epizootic Abortion, Contagious Abortion and Bang's disease. According to the Food and Agriculture Organization, the World Health Organization and the Office International des Epizooties, brucellosis is still one of the most important and widespread zoonosis in the world. The disease spreads mainly by the ingestion of unpasteurized dairy products. Officially approved methods of commercial pasteurization render naturally Brucella contaminated raw milk safe for consumption. However, most cases in humans are occupational and occur in farmers, veterinarians and butchers. The organism can be isolated from many organs other than the udder and uterus and the handling of a carcass of an infected animal may represent severe exposure (Radostits *et al*., 2000).

In humans, brucellosis can be caused by B. abortus, B. melitensis, B. suis biovars 1-4 and, rarely, B. canis or marine mammal Brucella. Live vaccines for B. abortus and B. melitensis, as well as the B. canis M- strain (a less virulent strain used as an antigen for serological testing), are also pathogenic for humans. B. ovis, B. neotomae and B. suis biovar 5 have not been linked to human disease (IOWA, 2007). In humans, Brucella abortus causes undulant fever; a disease characterized by intermittent fever, headaches, fatigue, joint and bone pain, psychotic disturbances, and other symptoms. It is contracted through exposure to Brucella abortus contaminated milk and infected organs from infected animals. Livestock and slaughter industry workers, and consumers of unpasteurized milk and other dairy products made from unpasteurized milk are at the greatest risk of contacting undulant fever. Transmission occurs through contact with the tissues of infected animals at slaughter or ingestion of unpasteurized milk derived from infected cattle (Richey and Harell, 1997).

2.2.6. Clinical Findings

The clinical findings are dependent upon the immune status of the herd. In highly susceptible non vaccinated pregnant cattle, abortion after the 5^{th} month of pregnancy is the typical feature of the disease in cattle. In subsequent pregnancies the fetus is usually carried to full term although second or even third abortions may occur in the same cow. Retention of the placenta and metritis are common sequelae to abortion. Mixed infections are usually the cause of the metritis which may be acute, with septicemia and death following, or chronic, leading to sterility. In the bull, orchitis and epididymitis occur occasionally. B. abortus can often be isolated from the tissues of non suppurative synovitis. Hygromatous swellings, especially of the knees, should be considered with suspicion (Radostits *et al*., 2000).

The incubation period of bovine brucellosis varies between 14 and 120 days. If an infection appears in a herd that has so far been immunologically naive, all pregnant animals will abort. However, if the infection is enzootic in a given herd, usually only firstly calving animals abort. The susceptibility of the animal depends significantly on their natural resistance (breed), their age, and their level of immunity and on environmental stress The transmission during mating is important but perhaps not as much as often supposed (Nicoletti, 1980). After infection of the regional lymph nodes, bacteraemia occurs which can last for 1-3 weeks and distribute the organisms to the lymphatic system, the large parenchyma and other organs and tissues. The facultative intracellular organisms may infect all organs and tissues and in pregnant animals, the uterus is a preferred site of infection where it leads to a necrotizing placentitis whereas in non pregnant animals, the first infection often occurs in the udder followed by the infection of the uterus later after the onset of pregnancy. In cattle, the uterus is the central site of multiplication of the pathogen, the enhanced virulence of the Brucellae inside the reproductive system is supposed to be the consequence of the increased level of the erythritol that is maintained in the reproductive system. A characteristic exudative and proliferative process develops in the gravid uterus starting from the epithelium of the villus of the chorion (Kassahun, 2003).

2.2.7. Diagnosis

Clinical diagnosis of brucellosis is not easily achieved. Laboratory testing is therefore very important for a correct identification of the disease in humans and for the detection and confirmation in animals. Definitive diagnosis is normally done by isolation and identification of the causative agent. While definitive, isolation is time-consuming, must be performed by highly skilled personnel, and it is hazardous. For these reasons, serological tests are normally preferred. Brucellosis serology has advanced considerably in the last decades with very sensitive and specific new tests available. Modern genetic characterization of Brucellae using molecular DNA technology has been developed. Several PCR-based assays have been proposed, from the rapid recognition of genus to differential identification of species and strains (Poester *et al*., 2011).

The diagnosis of the disease can be challenging and is frequently delayed or missed because the clinical picture may mimic other infectious and non-infectious conditions. Recently, ELISA has taken over as an important serological tool in the diagnosis of brucellosis because of its economy, sensitivity, specificity, rapidity, reproducibility, and easy interpretation through colorimetric end product (Patel *et al*., 2014). The diagnosis of brucellosis is usually performed by a combination of methods. A definitive diagnostic technique is not available yet, in spite of being pursued for more than one century (Poester *et al*., 2011).

The clinical illness is often nonspecific when considered in the individual patient. Therefore, evaluation of patients often includes a number of tests dictated by the differential diagnosis. When a patient is suspected of having brucellosis, at least one blood specimen, bone marrow and tissues aspirates can be taken for culture. Bone marrow cultures have been positive more often than blood cultures, especially when patients have taken antibiotics (Gotuzzo *et al*., 1986). The intracellular localization of Brucella within reticulo endothelial cells may account for the positive cultures from bone marrow aspirates at a time when blood cultures from the same patient are negative. Isolation of Brucella organisms provides the definitive diagnosis and isolation of organisms from the tissues of infected animals may also be important (Kassahun, 2003).

All abortions in cattle in late gestation, starting from the fifth month, should be treated as suspected brucellosis and should be investigated. The clinical picture is not pathognomonic, although the herd history may be helpful. Unequivocal diagnosis of Brucella infections can be made only by the isolation and identification of Brucella, but in situations where bacteriological examination is not practicable, diagnosis must be based on serological methods. There is no single test by which a bacterium can be identified as Brucella. A combination of growth characteristics, serological, bacteriological and/or molecular methods is usually needed (OIE, 2012).

Bacteriologic examinations are of less practical use in determining the prevalence rate of brucellosis on humans and cattle population. This is due to the long incubation period, higher cost and usual negative results of culture. Thus, the use of various standard serological testing methods like Rose Bengal Plate Test (RBPT), 2-mercaptoethanol Test (2ME), and Dipstick Assay (DSA) in humans and also Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT) are methods of choice in testing of animals (MacMillan, 1990). However, the serological tests should be accomplished in combined and justifiable manner to avoid false positive or negative results (Kassahun, 2003). No single serological test is appropriate in all epidemiological situations; all have limitations especially when it comes to screening individual animals (Godfroid *et al*., 2002; Nielsen *et al*., 2006). Consideration should be given to all factors that impact on the relevance of the test method and test results to a specific diagnostic interpretation or application. In epidemiological units where vaccination with smooth Brucella practiced, false-positive reactions may be expected among the vaccinated animals because of antibodies cross-reacting with wild strain infection (OIE, 2012).

2.2.8. Treatment

Brucellosis is one of the drug neglected diseases and treatment of brucellosis in domestic animals is not indicated. However, humans are usually treated with the following antibiotics, doxicycline with rifampicine. Relapses are, however, possible. Single agent therapy for brucellosis has now been abandoned because of the high rates of failure and relapse and the potential development of antibiotic resistance. Relatively short courses (less than 8 weeks) of treatment with antibiotic combinations have similarly been associated with high rates of relapse (Luzzi *et al*., 1993). The combination doxycycline and an aminoglycoside (gentamicin, streptomycin, or netlimicin) for 4 weeks followed by the combination of doxycycline and rifampin for 4 to 8 weekdays is the most effective regimen (Solera, 1995). The doxycycline /aminoglycoside combination is more effective than the doxycycline/rifampin combination in that rifampin reduces levels of doxcycline in plasma (Corbel, 1997).

Several chemotherapeutic agents have been employed in recent decades for the treatment of Brucella abortus infection in cows; however, none of these has been entirely successful. (Radwan *et* *al*., 1993) identified two therapeutic regimens that were effective in eliminating Brucellae from naturally infected cows. Each involved repeat treatments with long-acting oxytetracycline and streptomycin administered by intramuscular injection and intra mammary infusion for up to six weeks. Before treatment commenced, all cows were dried off (Ausvetplan, 2005).

2.2.9. Control and Prevention

The brucellosis control and eradication program has been and continues to be multi-faceted; the program uses surveillance testing at the farm, at the stock markets, and at slaughter facilities; quarantine and herd depopulation with indemnity payments; herd management; and vaccination. Any bovine that is known to be infected with the field strain of Brucella abortus is required to be placed under quarantine until slaughtered (Richey and Harell, 1997).

Although controlled or eradicated in a number of developed countries, re-introduction of brucellosis remains a constant threat, while in others, especially in the developing world, this disease continues to exert its devastating impact perpetuating poverty. Despite tremendous efforts and financial investments, many European Mediterranean countries have yet to eradicate this disease. Many factors, in particular the types of husbandry system, may have contributed to the failure to effectively control the disease in these countries. The reemergence of brucellosis as a major veterinary and public health problem in the former Soviet Republic during the past decade through a weakening of the veterinary system and transition from large government controlled farms to small-scale private farming further emphasizes the essential role of a continued and coordinated control effort. The transmission and spread of brucellosis is affected by a variety of factors and good knowledge of these is essential to the success of a control policy (Reviriego *et al*., 2000; Bikas *et al*., 2003; Minas *et al*., 2004). In general, prevalence of brucellosis usually is higher and control more problematic in pastoral or migratory populations, practiced by a significant proportion of the agricultural population of Africa. Vaccination of livestock is crucial to the control of brucellosis. Effective reduction of disease prevalence in livestock through mass vaccination eventually will also lead to a reduction of brucellosis in the human population. However, vaccination alone is not sufficient and should be accompanied by other measures such as restriction of animal movement and trade, culling of infected animals and improved farm sanitation to reduce the further spread of disease. In addition, a surveillance system is essential to control the efficacy of control measures and to identify outbreaks at an early stage. Clearly the control of brucellosis requires significant efforts both in terms of human and financial resources and time. In Argentina and other countries in South and Central America, brucellosis has been recognized as a disease problem since the 19^{th} century, but in spite of control efforts starting in Argentina in 1932, the disease still is not considered to be controlled in this country (Samartino, 2002). Despite the bleak situation outlined above, in resource poor countries control measures provided that they are adapted to the local situation and supported by the local population and instigated together with improved diagnostics, could provide immediate cost effective benefits (Roth *et al*., 2002). Demonstration of the cost-effectiveness of control measures is an essential prerequisite to gain acceptance and sustainability of such efforts. Veterinary vaccines for brucellosis are available for brucellosis in cattle and in small ruminants (Schurig *et al*., 2002). The attenuated live B. abortus S19 vaccine is the recommended vaccine for bovine brucellosis (Smits and Culter, 2004).

Vaccination of livestock is crucial to the control of brucellosis. Effective reduction of disease prevalence in livestock through mass vaccination will eventually lead to reduction of brucellosis in the human population (Henk *et al*., 2004). Until recently, "Strain-19 vaccine" was the only brucellosis vaccine used in the brucellosis control programs for cattle in the United States. Strain-19 vaccine was and still is an effective tool in brucellosis control. However, as with any tool, using Strain-19 vaccine has its advantages and disadvantages. Strain-19 is a live vaccine that stimulates the immune system of the vaccinated animal to resist a brucellosis disease challenge, produce antibodies against the disease organisms, and kill off the vaccine organisms. Normally, a vaccinated animal will retain the resistance to disease for an extended period of time (years) but the detectable antibodies will disappear in a few months (Richey and Harell, 1997).

3. MATERIALS AND METHODS

3.1. Study Area

Gondar town, the capital town of North Gondar Administrative zone is located in Amhara National Regional state 740 km away from Adiss Ababa to North West direction. The town is found at latitude of 12.3-13.8°N, at a longitude of 35.3-35.7°E and at 2200m a.m.s.l. The annual mean minimum and maximum temperature of the area vary between 12.3-17.7°c and 22-30°c respectively with an annual average temperature of 19.7°c. The region receives a bimodal rainfall, the average annual precipitation being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June to September (CSA, 2012). The agricultural practices observed in the area are cereal based crop production and livestock farming. The livestock population of North Gondar Zone is estimated to be 1936514 cattle, 524063 sheep, 682264 goats, 36,828 horses, 12473 mules, 223,116 donkeys and 3165068 poultry (MoARD, 2013).

3.2. Study population, sample size determination and sampling methods

Indigenous, cross and exotic breeds of cows which are calved and kept under extensive, semi-intensive and extensive husbandry system were randomly selected from the study area. Exotic breeds in this study indicates animals having >75% exotic blood level.

3.3. Study design

A cross sectional study was carried out in dairy cows on indigenous, exotic and cross-breeds using questionnaire survey and serological test RBPT. Farms and cows for this study were selected randomly. Sample size for serum collection was determined using the groups is according to the result formula given by Thrusfield (2005) expected prevalence of 50% at 95% confidence interval a sample size of 384 cows were sampled.

$N=1.962\times P_{exp}(1-P_{exp})/d^2$

Where, n=required sample size

P_{exp}=expected prevalence

d= desired absolute precision

$N=1.962^2*50/100(1-50/100)/(0.5)^2=384$

However, a total of 406 cattle were sampled to increase the probability of positive result as the expected prevalence is low in the study area.

3.4. Study methodology

Simple random sampling method was followed to select both the farms and study animals. Owners were interviewed for the presence of mainly abortion history of their cows, their husbandry system and way of breeding and other parameters. Then blood sample were collected from the cows selected randomly from their cows.

3.4.1. Questionnaires

A questionnaire survey on 47 farm owners was conducted to determine the prevalence of abortion and its relation with brucellosis sero-positivity. The questionnaire was designed to collect information on factors that are believed to be a risk factor for Brucella infection. This include breed, age of animal, parity and way of breeding were recorded. In addition the clinical indicators including history of abortion and RFM were interviewed and recorded.

3.4.2. Collection of Serum Samples

Blood samples were collected from the jugular vein of each selected animals using plain vacutainer tube and needle. Identification of each animal was labeled on corresponding vacutainer tubes and kept over-night at room temperature to allow clotting. At the next day sera were collected from the clot to another tube which animals /identification were coincided. Serum samples were kept at -20°C at University of Gondar Faculty of Veterinary Medicine Veterinary Microbiology Laboratory until tested using RBPT.

3.4.3. Serology Test

The Rose Bengal Plate Test (RBPT) was employed as a screening test on the serum samples for the presence of Brucella agglutinins. The protocol of RBPT as recommended by OIE was used as screening test to test the presence of Brucella antibody in the sampled sera. The RBPT is generally considered to be as a sensitive test (Dohoo *et al*., 1996) reported 97.9% sensitive for RBPT. The false positive results in the RBPT could be due to cross reactions with other bacteria such as *Yersinia enterocolitica*, *E. coli*, *Salmonella* species and *Pastuerella* species. The CFT is recognized as the most reliable diagnostic test to be used routinely for individual animals. It rarely exhibits non-specific reactions and does not work as the disease becomes chronic. The test was performed according to manufacturer's manual. Before performing test, antigen and sera were brought to room temperature. One drop (30µl) of serum was taken on a glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (30µl) of Rose Bengal antigen was added. The antigen and serum were mixed thoroughly with the spreader and then the slide was rotated for four minutes. The result was read immediately after four minutes.

3.5. Data management and analysis

All data collected during the study period was checked, coded and entered in to Microsoft Excel spreadsheet and analyzed using SPSS software version 16.0. The prevalence of abortion after 5^{th} month of pregnancy was calculated as the number of farms encountered abortion cases after 5^{th} month of pregnancy divided by the total number of farms studied. The sero-prevalence bovine brucellosis was calculated as the number of Rose Bengal test positive sera divided by the total number of sera samples. Pearson's chi-square (x^2) was used to evaluate the association of different variables with the prevalence of abortion after 5^{th} month of pregnancy and sero-prevalence of bovine brucellosis. P-value<0.05 (at 5% level of significance) was considered as statistically significant.

4. RESULT

4.1. Questionner Study Result

Out of 47 farms found in and around Gondar town the questionner data revealed that there is an overall prevalence of 10.6% (5 farms) abortion. Out of 47 farms in which the survey was conducted breed of animals, herd size of the farm, production system and breeding system of the farms were investigated as a risk factor for the prevalence of abortion. All risk factors have no statistically significance for the prevalence of abortion in this study. The relationship of risk factors with the prevalence of abortion is provided in Table 1.

Table 1: Summary of relationship of risk factors with prevalence of abortion.

Risk factors	Category	No of farms	Farms with abortion case	Prevalence (%)
Breed	Local	14	0	0.0
	Cross	22	4	8.5
	Exotic	3	0	0.0
	Mixed	8	1	2.1
	Total	47	5	10.6
Herd size	<10	26	4	6.4
	10-20	17	1	2.1
	>20	4	0	2.1
	Total	47	5	10.6
Production system	Intensive	21	4	8.5
	Semi-intensive	12	1	2.1
	Extensive	14	0	0.0
	Total	47	5	10.6
Breeding system	AI	11	3	6.4
	Natural mating	21	2	4.3
	Mixed	15	0	0.0
	Total	47	5	10.6

4.2. Rose Bengal Plate Test Result

Out of a total 406 serum samples tested 20 were positive in Rose Bengal Plate test with an overall prevalence of 4.9%.

The relationship between breeds and bovine brucellosis is provided in Figure 1. The maximum prevalence was recorded in cross breeds (4.2%) while minimum prevalence was observed in local breeds (0.2%). There was statistically significant difference between breeds.

Pearson's chi-square test (χ^2=10.645, p=0.005).

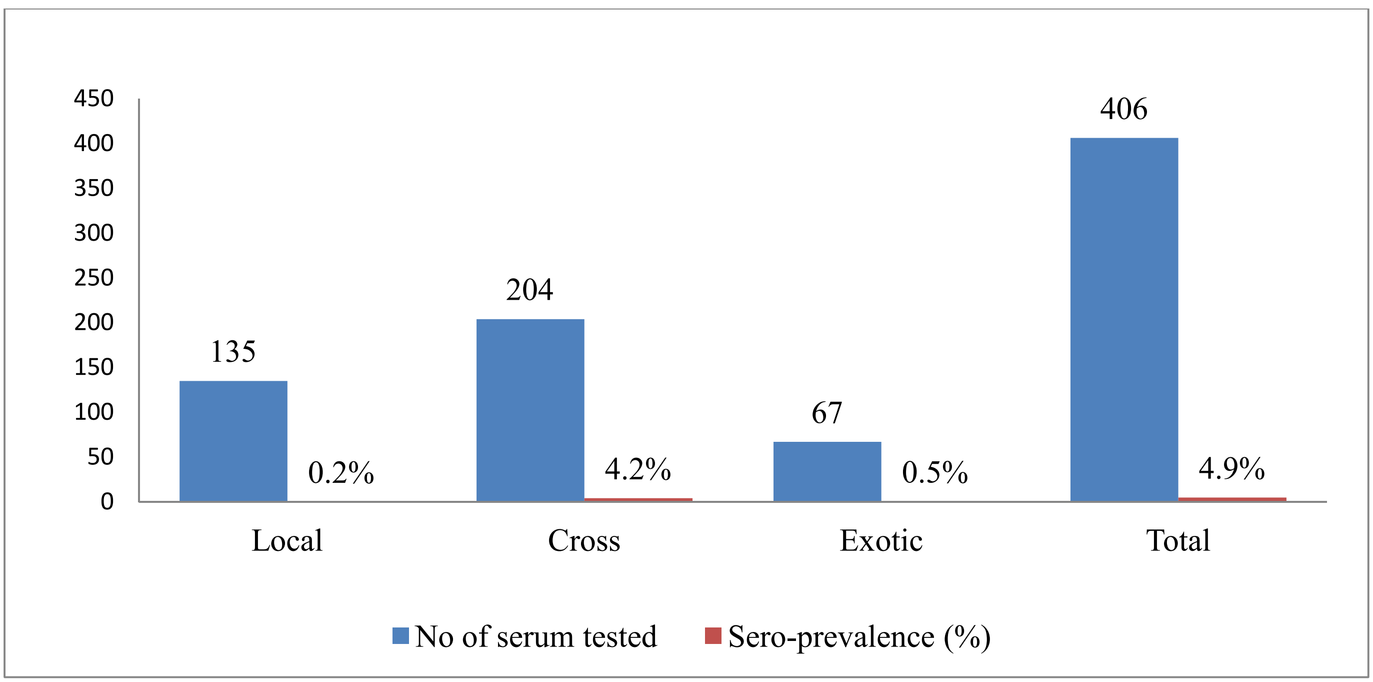

Figure 1: Sero-prevalence of bovine brucellosis on basis of breed.

The Sero-prevalence rate of bovine brucellosis between different parity groups of ≤2 calved, 2-5 calved and ≥5 calved cows was also investigated. The highest sero-prevalence was recorded in cows calved ≥5 calves (2.5%) and the lowest sero-prevalence recorded in cows calved ≤2 calves (0.5%) and there was statistically significant difference between parity groups.

Table 2: Sero-prevalence of bovine brucellosis on the basis of parity group.

Parity group	No of sera tested	Positive sera	Sero-prevalence (%)
≤2 calved	89	2	0.5
2-5 calved	223	8	2.0
≥5 calved	94	10	2.5
Total	406	20	4.9

Pearson's chi-square test (χ^2=8.766, p=0.012).

The sero-prevalence rate of bovine brucellosis between different production systems was also investigated. The highest sero-prevalence was recorded in cows kept under intensive production system (3.9%) and the lowest sero-prevalence recorded in cows kept under extensive production system (0.2%). There was statistically significant difference between different production systems.

Pearson's chi-square test (χ^2=6.612, p=0.037).

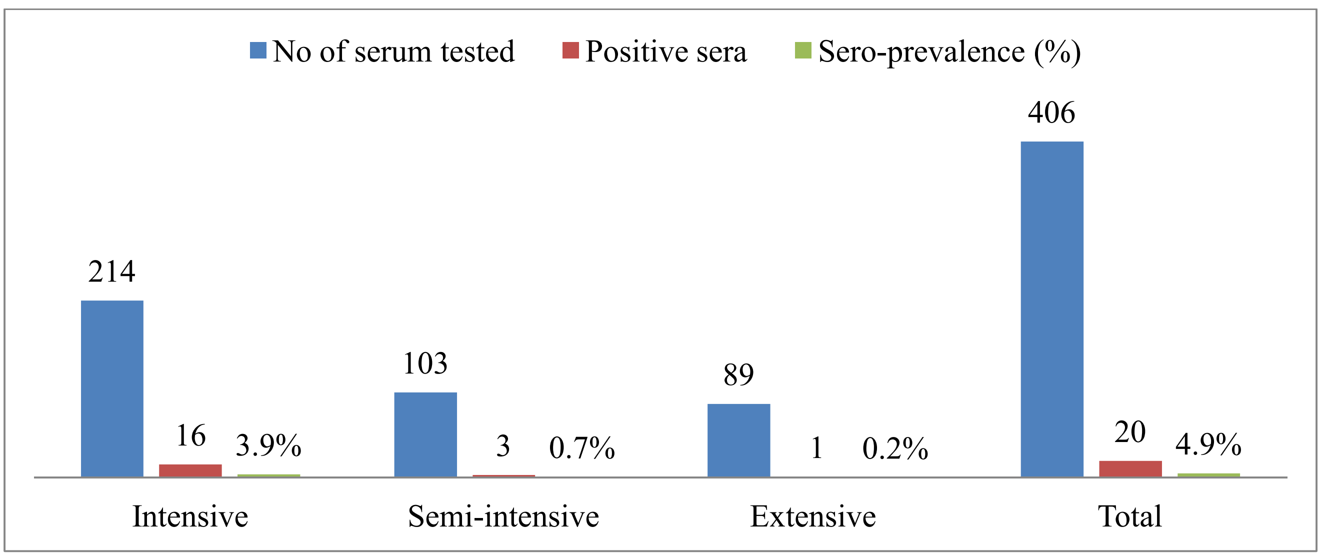

Figure 2. Sero-prevalence of bovine brucellosis on the basis of different production systems

The sero-prevalence rate of bovine brucellosis between different breeding systems was also investigated. The highest sero-prevalence was recorded in cows breed with artificial insemination (3.0 %) and the lowest sero-prevalence was recorded in cows breed with natural mating (0.7%) and there was statistically significant difference between different production systems.

Table 3: Sero-prevalence of bovine brucellosis on the basis of breeding system.

Breeding system	No of serum tested	Positive sera	Sero-prevalence (%)
AI	126	12	3.0
Natural mating	135	3	0.7
Mixed	145	5	1.2
Total	406	20	4.9

Pearson's chi-square test (χ^2=8.471, p=0.014).

The sero-prevalence rate of bovine brucellosis with the herd size of the farms where cows are kept was also investigated. The highest sero-prevalence was recorded in herd size groups of 10-20 animals (2.2%) and the lowest in herd size groups of ≥20 animals (1.0%). There was no statistically significant difference between different herd size groups.

Table 4: Sero-revalence of bovine brucellosis on the basis of herd size.

Herd size	No of serum tested	Positive sera	Sero-prevalence (%)
≤ 10 animals	149	7	1.7
10-20 animals	162	9	2.2
≥ 20 animals	95	4	1.0
Total	406	20	4.9

Pearson's chi-square test (χ^2=0.257, p=0.879).

The sero-prevalence rate of bovine brucellosis with the abortion history of cows was also investigated. The highest sero-prevalence was recorded in cows having abortion history after 5th month of pregnancy (3.2%) and the lowest in cows having no abortion history after 5th month of pregnancy (0.5%).There was statistically significant difference between abortion history of cows.

Table 5: Sero-prevalence of bovine brucellosis on the basis of abortion history.

Abortion history	No of serum tested	Positive sera	Sero-prevalence (%)
Before 5th month	41	2	0.5
After 5th month	58	13	3.2
No history	307	5	1.2
Total	406	20	4.9

Pearson's chi-square test (χ^2=45.000, p=0.000).

5. DISCUSSION

Bovine brucellosis is mainly characterized by abortion and retained fetal membrane. To know the prevalence of abortion at herd level in and around Gondar town, a questionner survey was taken though it is not possible to say all cases are due to brucellosis as other causes like management, feeding and other infectious problems may cause abortion. A total of 47 farms were selected randomly and a study was conducted. Out of 47 farms 5 had abortion history which is an overall herd prevalence of 10.6%. This questioner survey is in agreement with Adane (1998) who reported 11.8% in Wolaita zone of Southern Ethiopia, Abraha (2003) who reported 7.4% in Tigray regional state, Degefa (2011) who reported 8.3% in Arsi zone of Oromia regional state and Tadesse (2003) who reported 6.8% in North Gondar zone of North-Western Ethiopia. The difference in the report may be due to different factors like husbandry system, agro-climatic condition of the study areas. High abortion rate could be due to exposure to physical exercise, stress long distance to search water point and pasture area and competition for available feed resource and infection. Robert (1971) indicated that incidence of abortion more than 2 to 5% should be viewed seriously and efforts should be done to determine its cause. This questioner survey indicated that one cause may be bovine brucellosis and further study on sero-prevalence of bovine brucellosis has been conducted using Rose Bengal Plate test. The study doesn’t indicate that the real sero-prevalence of bovine brucellosis as the test is screening test. Confirmatory test was not conducted due to absence of the test in the study area. To determine the sero-prevalence complement fixation test should be used.

A total of 406 serum samples were collected from randomly selected animals found in and around Gondar town and a screening test was conducted using Rose Bengal Plate test and 20 samples were Rose Bengal Test positive. This indicates an overall screening sero-prevalence of 4.9%. Yohannes *et al.* (2012) reported that out of 406 samples tested 12 (2.96%) samples found RBPT positive 8 (1.97%) were confirmed as CFT positive. The report of this study is in agreement with Sheferaw, (1994) with 2.1% in Shoa region Berhe, *et al*. (2007) who reported 3.19 in Tigray region, Jergefa *et al*. (2009) who reported 2.9% in central Oromia, Scacchia (2009) 2.77% in Eriteria, Asmare *et al*. (2010) with prevalence of 1.92% in Sidama zone, Degefu (2011) who reported 1.84% RBPT and

1.38% CFT in Jijiga zone of Somalia regional state, Magersa *et al*. (2011) who reported 3.5% in south eastern Ethiopia, and Yohannes *et al*. (2013) who reported 2.96% RBPT and 1.97% CFT in Guto-Gida district of East Wollega zone. On the contrary, it is by far much lower than the previous reports of Molla (1989) who reported 7.62% in Arsi region, Rashid (1993) with 38.7% in cattle owned by institute of agriculture research farm, Shiferaw (1994) with 12.34% in and around Bahir Dar, Gebremariam (1996) with 18.4% in the dairy farms of around Addis Ababa. Other investigators, for instance Tadesse (2003) in north Gondar, Lidia (2008) in central highland, Tolosa (2008) in Jimma zone and Degefa (2011) in Arsi zone of Oromiya regional state reported 0.14, 0.77, 0.45% and 0.05%, respectively that indicates lower overall prevalence.

Out of 11 risk factors on general characteristics of dairy farms, only five (herd size, type of animals, type of breed, age of owner and knowledge gained by owners) showed significant ($p<0.05$) association with occurrence of bovine brucellosis. None of the risk factors on introduction of infection to farms and management systems of farms was found significantly associated with occurrence of brucellosis. Among risk factors on exposure of disease, history of abortion, retention of placenta, still birth and metritis/endometritis showed significant ($p<0.05$) association with prevalence of bovine brucellosis (Patel *et al*, 2014).

Among the potential risk factors considered in the present study, the breed of cattle was shown to have a significant effect on the serological prevalence rate of bovine brucellosis ($p < 0.05$), for both the overall study area though there is still controversy among different authors on the issue of breed susceptibility to brucellosis. In this study, the sero-prevalence was found to be higher in cross-bred animals (4.2%) than indigenous ones (0.2%), Jergefa *et al*. (2009) in their study found that breed of cattle has significant effect on the serological prevalence of brucellosis and is higher in cross-bred (10.3%) than in indigenous ones (2.7%) Jergefa *et al.* (2009). On the contrary Yohannes *et al.* (2012) reported that there is no statically significant difference on susceptibility of breeds for bovine brucellosis though the report showed higher prevalence in crosses breeds (3.64%) than in indigenous breeds (1.7%). This significant difference could be due to the compounded effects of management and mating methods. As observed in the present study, farmers who owned crossbred cattle tended to follow intensive management methods and prefer artificial insemination to natural mating for breeding.

The type of breeding system used by farmers was shown to significantly affect the prevalence of bovine brucellosis ($p<0.05$). A higher prevalence was encountered on animals that breed using artificial insemination (3.0%). This report is in agreement with Jergefa *et al.* (2009). Who reported a higher sero-prevalence of brucellosis was observed on farms that used artificial insemination (10.3%), as opposed to those that used natural mating (2.7%) and Adugna *et al*. (2013) who reported 2.6%. This could be the result of poor hygiene practices before and after insemination and inappropriate techniques. The inseminators had only a few months of training and there is no regular monitoring or upgrading of their skills. However, this area needs more detailed investigation.

Another important risk factor with a significant effect in the study area ($p < 0.05$), was the animal management system. In this study, a higher prevalence was observed in cattle under intensive production systems (3.9%) than in those under extensive farming (0.2%). This finding agreed with previous reports of Jergefa *et al*. (2009) who reported that more positive test results were recorded in animals raised under intensive production systems (10.3%) than in those raised in extensive systems (2.7%). However, it was not in accord with a study by Gebretsadik (2005) in northern Ethiopia. This study reported a higher prevalence rate in cattle under extensive management systems. The higher prevalence in intensive production systems could be explained by the fact that there is a greater chance of contact between infected and healthy animals in these systems, or between healthy animals and infectious materials, since most farmers do not follow hygienic practices. The higher prevalence in extensive management systems reported by Gebretsadik (2005) could be due to the transhumant nature of cattle herding in northern Ethiopia, which can enhance the spread and distribution of infection.

A higher prevalence was found in animals from herd size groups of 10-20 animals (2.2%) with ($p>0.05$). This report is in contrary with the report of Jergefa *et al*. (2009) who reported as in smaller herds (10.2%) than in animals from medium (2.8%) and large (2.2%) herds. The reason for the difference of report may be the absence of many large herd sized commercial farms in the study area.

The highest brucellosis sero-prevalence was observed within the older animals as measured by parity >5 calved (2.5%) and more than < 2 calved (0.5%) with ($p<0.05$). This report is in agreement with Kebede *et al.* (2008). Who reported 17.8% in >8 years old animals and 1.4% in <2 years old animals. This may be due to frequent exposure to risk factors like mating and infection due to contact.

In this study, brucellosis seropositivity differs between herds which experienced abortion and retained fetal membrane and those which did not. As abortion and retained fetal membrane are features of brucellosis, it is common to find such result. A previous history of abortion after 5^{th} month of pregnancy was, as expected, significantly associated with sero-positivity ($p<0.05$) and prevalence rate of 3.2%. Other studies have also shown a significant association between seropositivity and abortion and RFM like Asfaw *et al*. (1998), Berhe *et al*. (2007), Tolosa *et al*. (2008), Asmare *et al*. (2010), Ibrahim (2003) and Mekonen *et al*. (2010), Adugna *et al.* (2013). This could be explained by the fact that abortions or stillbirths and retained placenta are typical outcomes of brucellosis.

6. CONCLUSION AND RECOMMENDATIONS

The prevalence of abortion and the ser-prevalence of bovine brucellosis in this study were 10.6% and 4.9%, respectively. The results don't show the real prevalence of the diseases as abortion may be caused by other causes and the serological test used was RBPT which is screening test for brucellosis. Both studies showed that there is a slight increase in the prevalence of the disease than those very few reports reported in the study area. Both in the questioner survey and sero-prevalence study different risk factors were considered. A difference in the prevalence of abortion and sero-prevalence of bovine brucellosis was observed. In the questioner survey higher prevalence of abortion was observed in cross breed of animals that are kept under intensive management system and breed using artificial insemination. Unlike the serological study, higher prevalence of abortion was observed in small holder farms having herd size of less than 10 animals. In the survey all the risk factors were not statically significant ($p>0.05$) for the prevalence of abortion. Likewise in the serological study higher sero-prevalence was observed in cross breed animals kept under intensive production system and bred using artificial insemination. The prevalence was also higher in herd size group of 10-20 animals which is different from the questioner survey. Except herd size all factors were found statically significant ($P<0.05$). Abortion after 5th month of pregnancy was found statically strong significant ($p=0.00$) for the occurrence of bovine brucellosis. In general there are no organized studies that had been done at the study area; this study shows that there is an indication of an increase in the occurrence of the disease than the previous reports.

Based on the observations in this study the following recommendations are forwarded.

- ✓ Surveillance should be done to determine the foci of infection at the area and farm level so that bovine brucellosis control and prevention mechanism can be designed.
- ✓ Livestock Production systems should be managed in a way that reduces the transmission and dissemination of bovine brucellosis.
- ✓ Awareness should be created for the farm owners on the risk factors for the increase on bovine brucellosis.

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8. ANNEX

Annex 1. Test procedure for Rose Bengal Plate Test.

i) Bring the serum samples and antigen to room temperature (22 ± 4°C); only sufficient antigen for the day's tests should be removed from the refrigerator.

ii) Place 25–30 µl of each serum sample on a white tile, enamel or plastic plate, or in a WHO haemagglutination plate.

iii) Shake the antigen bottle well, but gently, and place an equal volume of antigen near each serum spot.

iv) Immediately after the last drop of antigen has been added to the plate, mix the serum and antigen thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter.

v) The mixture is agitated gently for 4 minutes at ambient temperature on a rocker or three-directional agitator (if the reaction zone is oval or round, respectively).

vi) Read for agglutination immediately after the 4-minute period is completed. Any visible reaction is considered to be positive. A control serum that gives a minimum positive reaction should be tested before each day's tests are begun to verify the sensitivity of test conditions.

Source; OIE Teristrial Manual, 2012

Annex 2. Questionarie to assess the Prevalence of Abortion in and Around Gondar Town

S.No……………

1. Code of the farm…………………………………….

2.Herd size…………………………………………………………………………………….

3. Number of fertile cows

4. Breed of cows A. Local B. Cross C. Exotic

5. Type of production A. Intensive B. Semi-intensive C. Extensive

6. Way of breeding A. Natural B. AI

7. Did any abortion case occurred in your farm? A. yes B. No

8. If yes, in which stage of pregnancy occurred A. <3 months B.3-5 months C 3.5-9 months

9. Did the abortion caused retained placenta? A. Yes B.no

10. What was the condition of the fetus during abortion?……………………………………………………………………………………… ………………………………………………………………………………………………… …………………………………………………………

11. Did the case occurred more than one time in one animal? A. Yes B. no

12. What is the current health status of the aborted cows? A. healthy B. died c. sterile

13. Which age group of animals are more frequently affected?

A. <3 years B.3-5 years C. 5-8 years D. >8years

14. Which Breeds of animals are more frequently affected? A. local B. Cross C. exotic

15. Which cows are more frequently affected? A. Cows breed through natural breeding B. Cows breed with AI

16. Any comment

………………………………………………………………………………………………… ……………………………………………………………………………

THANK YOU FOR YOUR RESPONSE.

Annex 3; Photographs of agglutination reaction of positive samples on RBPT

Annex 4; Photographs from some selected farms during sample collection.

9. DECLARATION

I the under signed, declare that the information presented here in my thesis is my original work has not been presented for degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Elias Alehegn Signature: ____________Date of submission:_________________

This thesis has been submitted for examination with my approval as university advisor.

Name: Dr. Shimels Tesfaye (DVM, MSc, Assistant Professor of Veterinary Microbiology)

Signature:_____________________